REPORT DOCUMENTATION

AD-A269 376

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	Interim March 1991 -- January 1993
	September 1993	

4. TITLE AND SUBTITLE
Effect of Radio-frequency Radiation (RFR) and Diazoluminomelanin (DALM) on the Growth Potential of Bacilli

5. FUNDING NUMBERS
PR - 2312
TA - W1
WU - 15

6. AUTHOR(S)
John G. Bruno
Jonathan L. Kiel

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Armstrong Laboratory
Occupational and Environmental Health Directorate
Radiofrequency Radiation Division
2503 D Street
Brooks Air Foce Base, Texas 78235-5102

8. PERFORMING ORGANIZATION REPORT NUMBER
AL-PC-1992-0011

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Published in the Proceedings of the First World Congress for Electricity and Magnetism in Biology and Medicine.

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*
DALM, which is thought to generate increased levels of free radicals in response to RFR, was synthesized in situ by Bacillus anthracis. Bacilli grown under DALM-producing conditions showed significantly lower plate counts in response to circularly polarized 2450 MHz RFR (100W/Kg) with exposures as short as 30 min. Bacilli grown on media for 3 days prior to irradiation demonstrated lower colony counts than 1 day old cultures under DALM-producing and nonproducing conditions. No significant RFR effects were observed for endospore preparations of bacilli grown in the absence of DALM substrates. Thus, DALM appears to function as a microwave-sensitive antibiotic which may operate through a free radical-mediated mechanism. Additionally, DALM-laden bacteria have demonstrated temperature-dependent "slow fluorescence," supporting the hypothesis that DALM toxicity is mediated by free radicals and suggesting that DALM may be of utility in the area of molecular electronics.

14. SUBJECT TERMS
Radiofrequency Radiation Effects, Bacterial Viability, Slow Fluorescence, Molecular Electronics

15. NUMBER OF PAGES
3

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

Martin Blank, Ed., *Electricity and Magnetism in Biology and Medicine*

EFFECT OF RADIO-FREQUENCY RADIATION (RFR) AND DIAZOLUMINOMELANIN (DALM) ON THE GROWTH POTENTIAL OF BACILLI

J. G. Bruno and J. L. Kiel*

Armstrong Laboratory, Brooks AFB, Tex.

DALM, which is thought to generate increased levels of free radicals in response to RFR, was synthesized in situ by *Bacillus anthracis*. Bacilli grown under DALM-producing conditions showed significantly lower plate counts in response to circularly polarized 2450MHz RFR (100 W/kg) with exposures as short as 30 min. Bacilli grown on media for 3 days prior to irradiation demonstrated lower colony counts than 1-day-old cultures under DALM-producing and nonproducing conditions. No significant RFR effects were observed for endospore preparations of bacilli grown in the absence of DALM substrates. Thus, DALM appears to function as a microwave-sensitive antibiotic that may operate through a free radical-mediated mechanism. In addition, DALM-laden bacteria have demonstrated temperature-dependent "slow fluorescence," which supports the hypothesis that DALM toxicity is mediated by free radicals and suggests that DALM may be of utility in molecular electronics.

Free radicals are known mediators of ionizing radiation bioeffects. At low levels, nonionizing radiation imparts insufficient energy to generate free radicals directly. Yet several investigators[1,2] have proposed or examined free-radical-driven biochemical processes to ascertain whether natural free-radical levels might be enhanced by RFR. Results indicated little evidence for microwave-induced cellular damage by free radicals secreted to the extracellular environment.[2] Hence, we reasoned that coupling RFR to a robust free-radical generator such as DALM, which is probably synthesized intracellularly or in the periplasmic space,[3] might amplify suspected bioeffects.

We initiated studies of *Bacillus anthracis* (nonpathogenic Sterne strain) under conditions thought to generate elevated levels of free radicals (i.e., DALM loaded) to determine whether nonthermal RFR-driven free-radical formation could kill bacteria. Bacilli were chosen for two reasons. First, one may examine RFR effects on purified, quiescent endospores (vaccine) as well as metabolically active vegetative cells. Second, when fed appropriate substrates (i.e., 3-amino-tyrosine, nitrate, and luminol), the bacteria synthesize a chemiluminescent polymer known as DALM.[3-5] DALM is thought to generate increased levels of free radicals in response to RFR, because its free radical-driven chemiluminescence increases with microwave heating.[5]

METHODS AND RESULTS

Figures 1(a) and (b) summarize results obtained from colony counts of bacteria cultivated for 1 day on TSA (trypticase soy agar) only, or TSA plus DALM substrates (320 mg/l 3-amino-L-tyrosine, 12 g/l potassium nitrate), and 100 mg/l 5-amino-2, 3-dihydro-1,4-phthalazinedione (luminol) with (solid line) or without (dashed line) RFR exposure. Similar results were obtained for bacteria cultured 3 days on TSA only ($n = 4, p = 0.0627$) and TSA plus DALM substrates ($n = 3, p = 0.0001$). Figure 1(c) illustrates that RFR had no appreciable effect on the growth potential of a live endospore vaccine (Thraxol-2, Mobay Corp., Shawnee, Kans.). These data were collected from samples irradiated with 2450MHz CW RFR for 30 min at 37 C in buffer consisting of sterile phosphate buffered saline (PBS), 0.75% $NaHCO_3$, and 0.03% H_2O_2, followed by serial 10-fold dilution in PBS and plating in liquid TSA cooled to 45 C. Controls (-RFR) were kept in a 37 C water bath to mimic temperature conditions during RFR exposure. All cells were initially diluted to an absorbance of 2.0 at 600 nm. Temperature was maintained at 37 C by means of a computer-controlled air cooling-heating system (Questech, San Antonio, Tex.). Colony counts greater than 300 were scored as too numerous to count, but assigned a value of 300 for plotting and statistical analysis. All p values were

*Correspondence to: J. L. Kiel, Armstrong Laboratory (AFSC), Brooks AFB TX 78235-5000

derived from 2-way analysis of variance (ANOVA). Subsequent data (not shown) suggest extinction of DALM-loaded bacteria somewhere between 4 and 8 h of RFR exposure.

SUMMARY AND CONCLUSIONS

This work demonstrates the microwave-augmented antibiotic effect of DALM. In addition, observations of reduced colony counts for DALM-laden bacilli and the lack of an RFR effect on quiescent endospores suggests that RFR-induced cellular damage is mediated by free radicals. The identity of free radicals generated by DALM is still unknown; however, we suspect the formate radical is a primary damaging species. Other radicals, such as the semiquinone, may also be involved, but probably to a much lesser extent. Global assessment of macromolecules from exposed and unexposed bacteria by use of silver-stained SDS polyacrylamide gels has revealed no obvious evidence of free-radical damage (data not shown). Only age-related and buffer-related changes (i.e., the carbonate/bicarbonate concentration was varied) in banding patterns are evident.

Although it is difficult to produce tangible evidence of free-radical damage by the methods employed here, three lines of evidence point to a free-radical mechanism (data not shown). First, only DALM-laden bacteria die or are growth inhibited in response to microwave exposures. Second, DALM-laden bacterial chemiluminesce strongly in an RF field, even at 37 C. This chemiluminescence is believed to originate from free-radical interaction with luminol moieties in DALM. Third, such bacteria exhibit the little-known phenomenon of "slow fluorescence" (unpublished observation).[6,7]

Slow fluorescence is a process distinct from both fluorescence and phosphorescence in that the emission is relatively long lived, but proceeds from an excited triplet (in this case possibly a semiquinone radical).[7] We have observed both red and green thermally dependent fluorescence emissions of a relatively long-lived nature that are quickly regenerated in DALM-loaded bacilli upon subsequent excitation. The observation of slow fluorescence strongly suggests the possibility of free-radical generation by DALM.

Thus, these experiments suggest that DALM, biosynthesized in situ, in conjunction with microwave energy may be used as a potentially effective antibiotic and photonic switching device (with the microwave field turning on the emission). Future work will focus on establishing the postulated free radical-mediated mechanism by addition of various free radical scavengers to abolish or abrogate the differences in colony counts reported here.[8]

REFERENCES

1. D. N. Erwin, *Aviation Space Environ. Med.* 59(11, Suppl.): A21-31, 1988.
2. J. L. Kiel and D. N. Erwin, *Int. J. Hyperthermia* 2: 201-212, 1986.
3. J. L. Kiel, J. E. Parker, J. L. Alls, and R. A. Weber, *Proc. Int. Conf. of IEEE-MBS* 13: 1605-1606, 1991.
4. J. L. Kiel, G. J. O'Brien, J. Dillon, and J. R. Wright, *Free Rad. Res. Comm.* 8: 115-121, 1990.
5. J. L. Kiel, C. Gabriel, D. M. Simmons, D. N. Erwin, and E. H. Grant, in J. L. Kiel, J. E. Parker, J. L. Alls, and R. A. Weber, *Proc. Int. Conf. of IEEE-MBS* 12: 1689-1690, 1990.
6. J. March, *Advanced Organic Chemistry*, New York: McGraw-Hill, 1977, p. 218.
7. C. C. Felix, J. S. Hyde, and R. C. Sealy, *Biochem. Biophys. Res. Comm.* 88: 456-461, 1979.

8. This work was supported by the Air Force Office of Scientific Research. The work was performed while Dr. Bruno held a National Research Council-AFSC Research Associateship.

FIG. 1

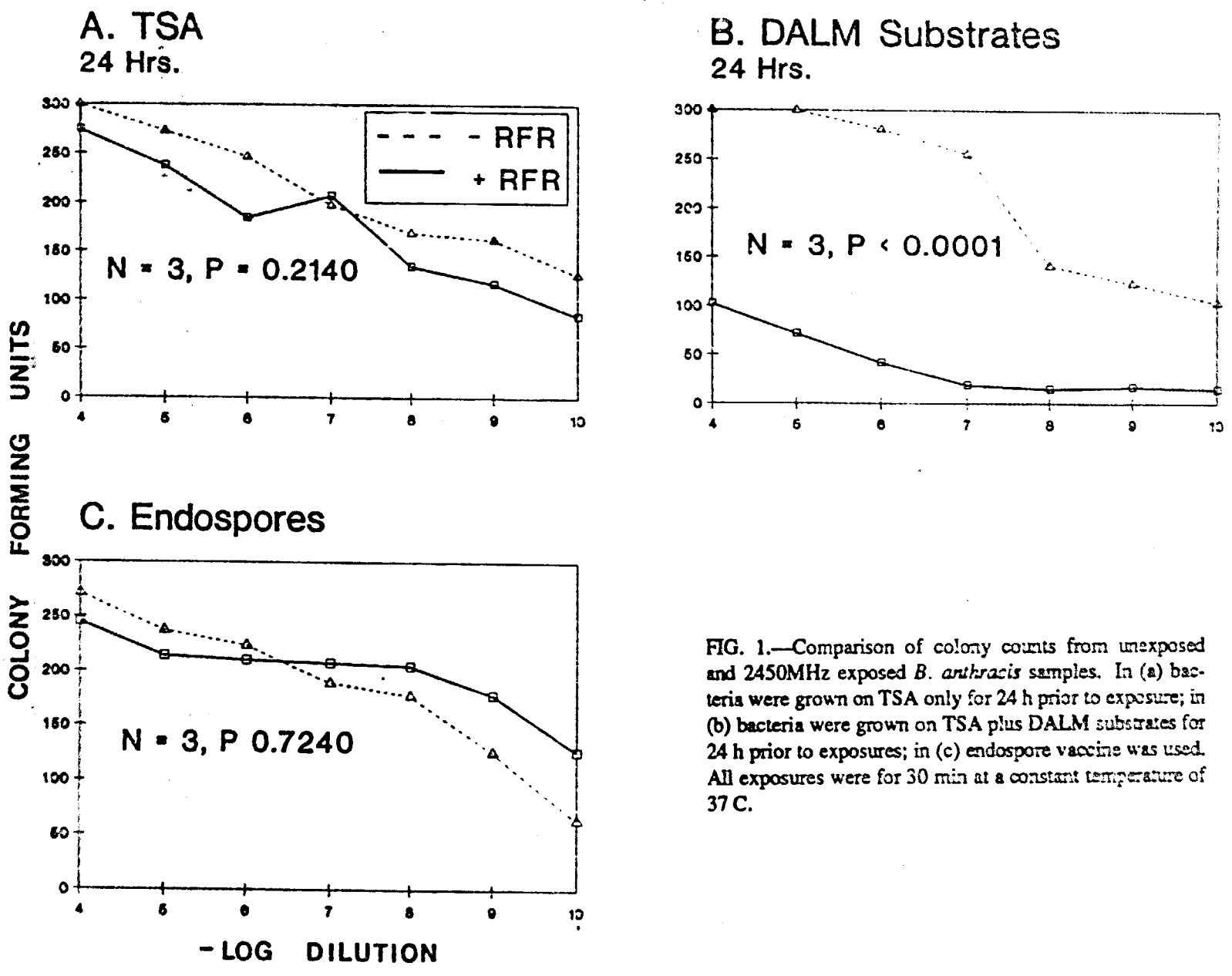

FIG. 1.—Comparison of colony counts from unexposed and 2450MHz exposed *B. anthracis* samples. In (a) bacteria were grown on TSA only for 24 h prior to exposure; in (b) bacteria were grown on TSA plus DALM substrates for 24 h prior to exposures; in (c) endospore vaccine was used. All exposures were for 30 min at a constant temperature of 37 C.